HIV

Dynamic T cells

In vivo studies of T-cell homeostasis in humans have, until now, been hindered by the lack of available tools that are safe for human use. But now, reporting in *The Journal of Clinical Investigation*, Hellerstein and colleagues use a highly innovative technique to define the dynamics of T-cell proliferation *in vivo* during HIV-1 infection.

The T-cell pool can be divided into populations that are functionally and kinetically distinct: memory cells have a long life span and high proliferative capacity; by contrast, effector cells typically die quickly by activation-induced cell death. The size of the T-cell pool is mainly regulated and maintained by proliferation of long-lived progenitor cells. In patients infected with HIV, a shortened average life span of T cells has been previously described. However, it remains controversial whether this results from direct cell killing by HIV or indirect effects of chronic activation. Without the benefit of cell-type specific markers that distinguish long- and short-lived cells, Hellerstein *et al.* developed an alternative approach to characterize the variation in life span in the memory/effector T-cell pool. By administration of deuterium — a safe, stable, non-radioactive isotope of hydrogen — in the form of deuterated water and deuterated glucose, which is incorporated into the DNA of dividing cells, the authors were able to measure T-cell kinetics *in vivo* over long periods of time. Long-term incorporation of deuterated water into DNA indicated that effector/memory T-cell subpopulations, but not naive T cells, had biphasic kinetics, consistent with the presence of T-cell subpopulations in humans with different life spans. This technique also enabled the detection in normal individuals of long-lived quiescent T cells that did not divide over the 9 week deuterium administration, probably representing the reservoir of progenitor cells.

They then went on to show that individuals with advanced HIV-1 infection had higher proportions of T cells that were short lived, in both the $CD4^+$ and $CD8^+$ memory/effector T-cell subpopulations, compared with healthy controls. These results were confirmed by analysis of die-away kinetics after short-term labelling with deuterated glucose, and together with long-term kinetic analysis, pool sizes and turnover rates of kinetically distinct subpopulations of T cells were calculated. In advanced HIV-1 infection, total pool sizes of short-lived cells were only moderately affected, whereas the

SIGNALLING

New link in the chain

After recognition of microbial products by members of the Toll-like receptor (TLR) family, the adaptor molecule MYD88 initiates intracellular signalling cascades that result in the activation of host defence mechanisms. However, recent studies have shown a MYD88-independent signalling pathway downstream of TLR3 and TLR4 that is regulated by the adaptor molecule TRIF (also known as TICAM1) and leads to the activation of interferon regulatory factor 3 (IRF3), as well as signals that sustain activation of nuclear factor-κB (NF-κB). Now, three independent studies have identified TRAM (also known as TICAM2) as an adaptor molecule that is a key link in the TRIF-dependent TLR4, but not TLR3, signalling cascade.

Previously identified TLR adaptor molecules contain Toll–interleukin 1 receptor (TIR) domains and each of the three groups identified TRAM using database searches for new TIR-domain-containing molecules. Oshiumi *et al.* and Fitzgerald *et al.* used a yeast two-hybrid assay and/or co-immunoprecipitation studies to show that TRAM interacts directly with both TRIF and TLR4, but not TLR3, implicating TRAM as a specific component of the TLR4-signalling cascade.

To determine the role of TRAM in TLR4 signalling, all three groups used the same approach — analysing the effect of ectopic overexpression of TRAM — and all observed activation of IRF3 and NF-κB, as well as activation of the promoter for the gene encoding interferon-β (IFN-β).

Oshiumi *et al.* and Fitzgerald *et al.* went on to generate a series of TRAM mutants, which they used to show that TRAM is crucial for IRF3 and NF-κB activation after ligation of TLR4 by lipopolysaccharide (LPS), but not TLR3 triggering with double-stranded RNA. Further evidence for the importance of TRAM in the TLR4-signalling pathway was provided by their demonstration that marked reduction of the level of TRAM by small interfering RNA impaired IRF3 and NF-κB activation in response to TLR4 triggering, but not TLR3 ligation.

Yamamoto *et al.* examined the physiological role of TRAM in the TLR-signalling cascade by generating Tram-knockout mice. Tram-deficient cells responded normally to signalling through TLR3; however the production of pro-inflammatory cytokines and IFN-β was substantially impaired after LPS triggering of TLR4, as was the activation of B cells. These functional defects coincided with inefficient activation of signalling molecules downstream of TRAM, including IRF3, and an inability to sustain NF-κB activation. By contrast, activation of the MYD88-signalling pathway was intact in the Tram-deficient cells. So, *in vivo,* TRAM is essential for the MYD88-independent signalling cascade after LPS ligation of TLR4. This cascade induces B-cell activation and the production of IFN-β; however, TRAM and MYD88 signals are required for the secretion of pro-inflammatory cytokines in response to LPS.

These studies provide invaluable insight into the TRIF-regulated MYD88-independent response generated after triggering of both TLR3 and TLR4. Ligation of these molecules induces distinct host responses and this specificity might be a result of TLR4 using the adaptor molecule TRAM to initiate signalling through TRIF, whereas TLR3 signals directly to TRIF.

Karen Honey

References and links

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size of the long-lived pool was reduced by a factor of more than four. This indicates that patients with HIV-1 have a reduced ability to generate long-lived progenitor cells for maintaining the T-cell pool. Effective treatment with antiretroviral therapy was able to restore the capacity to generate these progenitor cells, by reducing the levels of T-cell proliferation and T-cell death in infected individuals.

These data provide evidence against a model in which the characteristic depletion of $CD4^+$ T cells that occurs in HIV-1 infection results from direct HIV-mediated cell killing, as long-lived progenitors of both $CD4^+$ and $CD8^+$ T-cell subpopulations were reduced in patients infected with HIV-1. Moreover, they favour a model in which increased levels of proliferation of effector/memory T cells in HIV infection reflect chronic activation.

Understanding how infection with HIV-1 affects T-cell homeostasis has implications for vaccine design as well as potential new therapeutic strategies.

Lucy Bird

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TOLERANCE

AIRE control in the thymus

The autoimmune regulator (AIRE) has been shown to regulate the ectopic expression of peripheral tissue-restricted antigens in thymic medullary epithelial cells — a process that is thought to be important for establishing central tolerance. However, the signals that control the expression of this transcription factor in the thymus are unknown. Work by Chin *et al.* now published in *Nature Immunology* shows an essential role for lymphotoxin (LT)-signalling pathways in controlling AIRE expression.

Mutations in *AIRE* result in autoimmune polyendocrinopathy candidiasis ectodermal dystrophy — a disorder characterized by the presence of organ-specific autoantibodies and multiorgan autoimmune destruction. Similarities between the phenotypes of $Aire^{-/-}$ mice and animals deficient for LTα or LTβ receptor (LTβR) in terms of peripheral organ lymphocyte infiltration led the authors to investigate the possible link between these pathways.

Real-time polymerase chain reaction showed that the levels of Aire messenger RNA were markedly reduced in thymi from $LT\alpha^{-/-}$ or $LT\beta R^{-/-}$ mice, as were the levels of the peripheral tissue-specific protein insulin. The thymic levels of Aire and insulin mRNA specifically and rapidly increased after injections with an agonist LTβR-specific antibody (3C8), showing that LTβR signalling can induce the expression of Aire and ectopic peripheral antigens.

To establish that it is thymic medullary epithelial cells that are responding to the LTβR signalling, Chin *et al.* stimulated a thymic stromal cell line with 3C8. The levels of Aire and insulin mRNA specifically increased following this treatment, indicating that LT signals mediated by medullary epithelial cells regulate Aire expression in the thymus.

Do the reduced levels of expression of Aire and insulin in the $LT\alpha^{-/-}$ or $LT\beta R^{-/-}$ mice lead to a breakdown in tolerance? At ~5–7 months of age, increased levels of insulin-specific antibodies were detected in these mice and generalized autoimmune disease developed. To confirm that this autoimmunity developed due to a defect in the lymphoid compartment, the authors transferred splenocytes from $LT\beta R^{-/-}$ or wild-type mice into irradiated recombination activation gene 1-deficient mice. Symptoms of autoimmunity developed in the recipients of $LT\beta R^{-/-}$ cells, but not in mice that received wild-type cells.

So, disruption of LT signalling in the thymus, which prevents Aire-mediated upregulation of ectopic peripheral genes, can lead to the disruption of central tolerance and the development of autoimmunity.

Jenny Buckland

References and links

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